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Storage of Serum or Whole Blood Samples? Effects of Time and Temperature on 22 Serum Analytes

By *Michael Heins*[1], *Wolfgang Heil*[2] and *Wolfgang Withold*[1]

[1] *Institut für Klinische Chemie und Laboratoriumsdiagnostik der Heinrich-Heine-Universität Düsseldorf, Germany*
[2] *Zentrallaboratorium Klinikum Wuppertal-Barmen, Universität Witten/Herdecke, Germany*

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Summary: Information on the stability of serum analytes during storage of serum or whole blood samples is often incomplete and sometimes contradictory. Using a widely available analyser (Hitachi 737/Boehringer), we therefore determined the effects of storage time and temperature on the measured concentrations of the following serum analytes: sodium, potassium, calcium, chloride, inorganic phosphate, magnesium, creatinine, urea, uric acid, bilirubin, cholesterol, HDL- and LDL-cholesterol, triacylglycerols, creatine kinase, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, alkaline phosphatase, α-amylase, lactate dehydrogenase and cholinesterase.

When separated serum was stored at + 9 °C for seven days, the mean changes in inorganic phosphate and lactate dehydrogenase exceeded significantly ($p < 0.05$ or 0.001, respectively) the maximum allowable inaccuracy according to the Guidelines of the German Federal Medical Council; all other quantities were sufficiently stable.

In serum at room temperature, inorganic phosphate, uric acid, HDL-cholesterol and triacylglycerols increased continuously, whereas bilirubin, LDL-cholesterol, creatine kinase and aspartate aminotransferase decreased more than the guidelines permit during the storage period ($p < 0.05$ for aspartate aminotransferase, $p < 0.001$ for the other analytes mentioned).

In whole blood stored for 7 days at + 9 °C, only the following serum analytes satisfied the stability requirements of the guidelines: calcium, urea, cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerols, creatine kinase, γ-glutamyltransferase and cholinesterase. When stored at room temperature, only sodium, uric acid, bilirubin, cholesterol, triacylglycerols, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, α-amylase and cholinesterase were still stable after 3 days.

The data collected show that all quantities examined are sufficiently stable for four days in separated serum stored at + 9 °C.

Introduction

A general problem in clinical laboratories is the stability of analytes during sample storage. Analytical methods have been continuously optimised to minimise the influence of disturbing factors. In earlier studies, information on the stability of analytes in serum and whole blood was based on methods, which have since become obsolete (1–5). In addition, the extent of interference by haemolysis strongly depends on the methods employed and the various modifications of a given method. Therefore we determined the effects of storage time and temperature on the measurement of the following serum analytes: sodium, potassium, calcium, chloride, inorganic phosphate, magnesium, creatinine, urea, uric acid, bilirubin, cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerols, creatine kinase (EC 2.7.3.2), aspartate aminotransferase (EC 2.6.1.1), alanine aminotransferase (EC 2.6.1.2), γ-glutamyltransferase (EC 2.3.2.2), alka-

line phosphatase (EC 3.1.3.1), α-amylase (EC 3.2.1.1), lactate dehydrogenase (EC 1.1.1.27) and cholinesterase (EC 3.1.1.8).

Materials and Methods

Apparatus

HDL- and LDL-cholesterol were determined manually using a spectral line photometer 6118 (Eppendorf). Magnesium was determined with an Hitachi 704 analyser (Boehringer) with batchwise processing of samples, while the other quantities were determined in random access made with an Hitachi 737 analyser (Boehringer). A spectrophotometer Lambda 5 (Perkin Elmer) was used for the spectrophotometric determination of free haemoglobin.

Reagents

The reagents and methods used are shown in table 1. Free haemoglobin in serum was determined spectrophotometrically according to *Harboe* (6).

Samples

Venous blood samples (210 ml) were drawn at 8.00 a.m. from 20 fasting, apparently healthy subjects in sitting position. For this purpose, 10 ml Vacutainer® sample tubes with polyester separation gel were used. These blood specimens were then randomised. Eleven of the blood samples were centrifuged immediately for 10 minutes at 3400 *g* and one of the sera obtained was used for measurement of the aforementioned quantities. Five serum tubes and 5 whole blood tubes were stored at room temperature (at 23–27 °C in a dark cupboard) and the remaining 5 of each, at 9 °C in a dark refrigerator. Centrifugation of the whole blood samples was

Tab. 1 Reagents and methods used in this study

Analyte	Method	Catalogue Number (Reagent 1 and 2)	Inter-assay-imprecision (%)	Maximum allowable inaccuracy (%)	Day zero (100% value of figures 2–5)
Sodium	Indirect potentiometry (Boehringer Mannheim GmbH, Mannheim, Germany)	*	0.7	2.0	139.0 mmol/l
Potassium	Indirect potentiometry (Boehringer)	*	1.3	2.7	3.92 mmol/l
Calcium	*o*-Cresolphthalein complexone (Boehringer)	1127551 and 1127650	1.4	3.3	2.28 mmol/l
Chloride	Indirect potentiometry (Boehringer)	*	1.3	2.0	106.6 mmol/l
Inorganic phosphate	Ammonium molybdate (Boehringer)	836281	2.0	5.0	1.33 mmol/l
Magnesium	Calmagite (Sigma Chemical Co., St. Louis [U.S.A.])	595-A	3.7	4.0	0.79 mmol/l
Creatinine	Kinetic modification of the *Jaffé* method (Boehringer)	1127918 and 1127926	3.1	6.0	91.1 µmol/l
Urea	Enzymatic kinetic UV test (Boehringer)	1127918 and 1127926	1.6	8.0	5.12 mmol/l
Uric acid	Enzymatic colorimetric test (Peridochrom©) (Boehringer)	791679 and 120927	2.5	6.0	281.1 µmol/l
Bilirubin	Dichlorphenyl diazonium method (Boehringer)	1127535 and 1127543	3.5	7.0	9.49 µmol/l
Cholesterol, total	Enzymatic colorimetric test (CHOD-PAP method) (E. Merck, Darmstadt, Germany)	14164, 14165, 14166 and 14167	1.8	6.0	5.05 mmol/l
HDL-cholesterol	Precipitation with phosphotungstic acid/ $MgCl_2$ (Merck)	14210, 14164–67	3.0	[6.0]	1.52 mmol/l
LDL-cholesterol	Precipitation with heparin at pH 5.12 (Merck)	14992, 14164–67	4.7	[6.0]	3.10 mmol/l
Triacylglycerol	Enzymatic colorimetric test (GPO-PAP method) (Boehringer)	1128027 and 1201301	2.0	7.0	1.25 mmol/l
Creatine kinase	Optimised standard method** at + 25 °C (Boehringer)	1127586 and 1091077	1.6	8.0	43.3 U/l
Aspartate aminotransferase	Optimised standard method** at + 25 °C (Boehringer)	1127802 and 1127829	2.4	7.0	9.10 U/l
Alanine aminotransferase	Optimised standard method** at + 25 °C (Boehringer)	1127837 and 1127845	1.8	7.0	7.68 U/l
γ-Glutamyl-transferase	According to *Szász* new at + 25 °C (Boehringer)	1127853 and 1127861	2.6	7.0	9.80 U/l
Alkaline phosphatase	Optimised standard method** at + 25 °C (Boehringer)	1127454 and 791377	2.7	7.0	100.4 U/l
α-Amylase	PNP method at + 25 °C (Boehringer)	1209035 and 791393	1.1	[7.0]	72.5 U/l
Lactate dehydrogenase	Optimised standard method** at + 25 °C (Boehringer)	1127969 and 1127977	1.7	7.0	143.1 U/l
Cholinesterase	Butyrylthiocholine iodide at + 25 °C (Boehringer)	1127667 and 1127675	1.7	7.0	5082.2 U/l

* Ion-selective electrode of Hitachi 737
** according to the Recommendations of the German Society of Clinical Chemistry
For the numbers in brackets there are no limits defined. The limits were derived from other enzymes or lipoproteins.

performed on the day of analysis. After 1, 2, 3, 4 and 7 days, measurements of the quantities examined were repeated. To avoid evaporation, sample tubes were kept closed until analysis.

Statistical analysis

The *Friedman* test was used to examine the influence of storage time on the measurable quantities. The changes of the values after storage were calculated for each subject as percentages of the initial values. The mean and standard deviation was computed and plotted. SPSS for windows was used to compute the Friedman test and Lotus 1-2-3 for OS/2 to calculate the percentage deviation.

Results

Release of haemoglobin

The release of free haemoglobin in whole blood samples of 10 apparently healthy persons stored at 9 °C in a refrigerator and at room temperature in a dark cupboard is shown in figure 1.

Storage of serum and whole blood

Significant changes in mean concentration or mean catalytic concentration during storage, which exceeded the maximum inaccuracy allowed by the Guidelines of the German Federal Council (7), were shown by the following quantities:

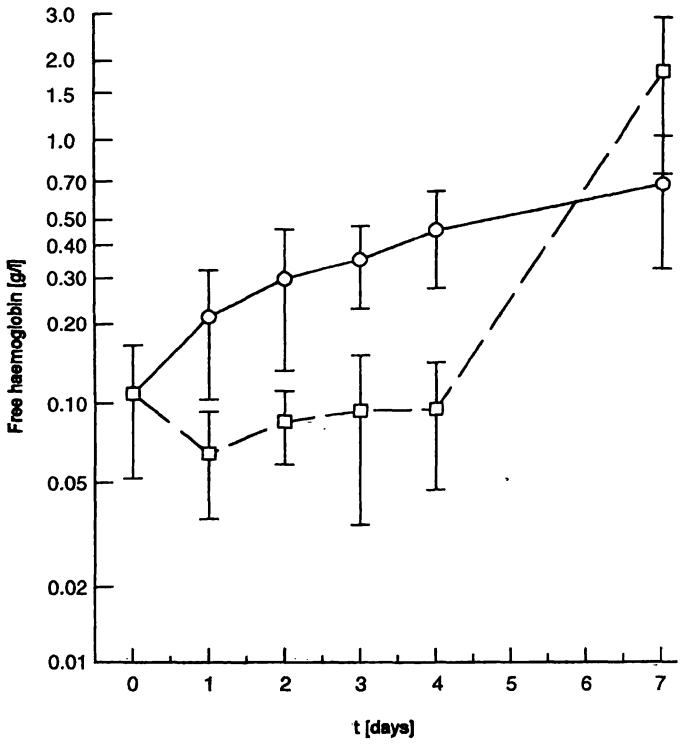


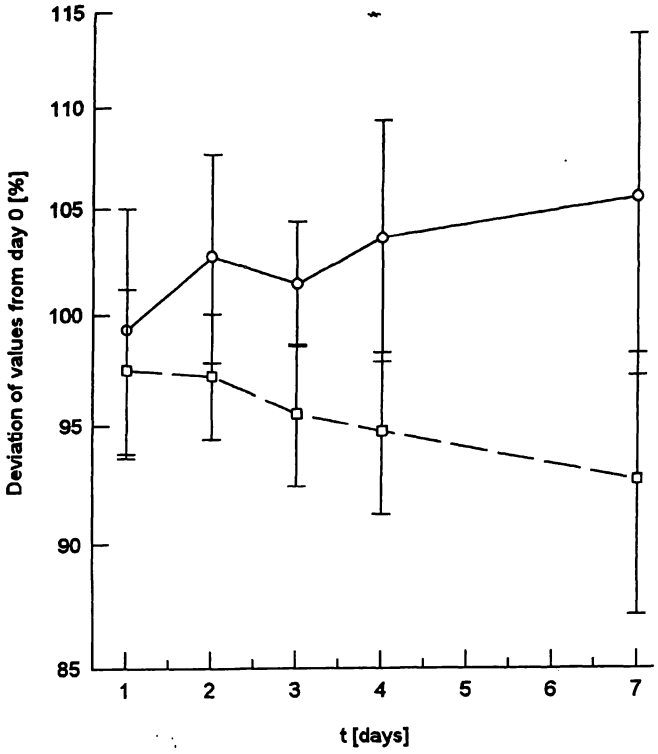
Fig. 1 Release of free haemoglobin during storage (circle at 9 °C, square at room temperature) in clotted blood (n = 10). Values are given in mean and standard deviation.

Analyte, condition	Change, time
Serum at 9 °C	
Inorganic phosphate	(increase after 7 days)
Lactate dehydrogenase	(decrease after 7 days)
Serum at room temperature	
Inorganic phosphate	(increase after 2 days)
Uric acid	(increase after 3 days)
Bilirubin	(decrease after 3 days)
HDL-cholesterol	(increase after 3 days)
LDL-cholesterol	(decrease after 2 days)
Triacylglycerols	(increase after 2 days)
Creatine kinase	(decrease after 3 days)
Aspartate aminotransferase	(decrease after 7 days)
Whole blood at 9 °C	
Sodium	(decrease after 1 day)
Potassium	(increase after 1 day)
Chloride	(decrease after 2 days)
Inorganic phosphate	(increase after 4 days)
Magnesium	(increase after 7 days)
Creatinine	(decrease after 1 day)
Uric acid	(increase after 3 days)
Alkaline phosphatase	(decrease after 1 day)
α-Amylase	(decrease after 2 days)
Lactate dehydrogenase	(increase after 1 day)
Whole blood at room temperature	
Sodium	(decrease after 7 days)
Potassium	(increase after 1 day)
Calcium	(decrease after 3 days)
Chloride	(decrease after 1 day)
Inorganic phosphate	(increase after 1 day)
Magnesium	(increase after 1 day)
Creatinine	(increase after 2 days)
Urea	(increase after 2 days)
HDL-cholesterol	(increase after 3 days)
LDL-cholesterol	(decrease after 2 days)
Alanine aminotransferase	(decrease after 7 days)
Alkaline phosphatase	(decrease after 7 days)
α-Amylase	(decrease after 7 days)
Lactate dehydrogenase	(increase after 1 day)

The changes in the measurable quantities are shown in percentage of the initial value in figures 2–5.

Discussion

Several phenomena were observed during sample storage. In serum, some analytes showed a decrease, others an increase due to enzymatic cleavage of precursor molecules. Prolonged contact of serum with red cells resulted in an exchange of substances between serum and the erythrocytes, which can cause dilution or can



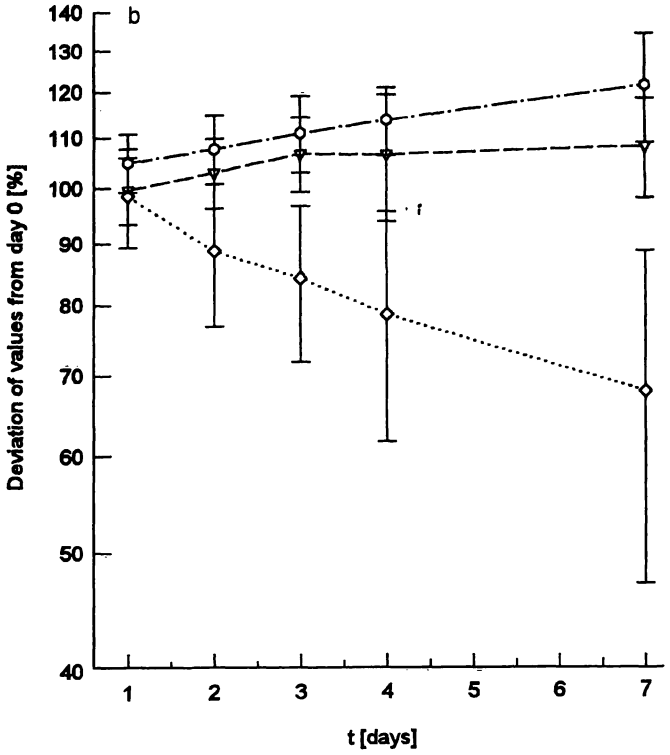
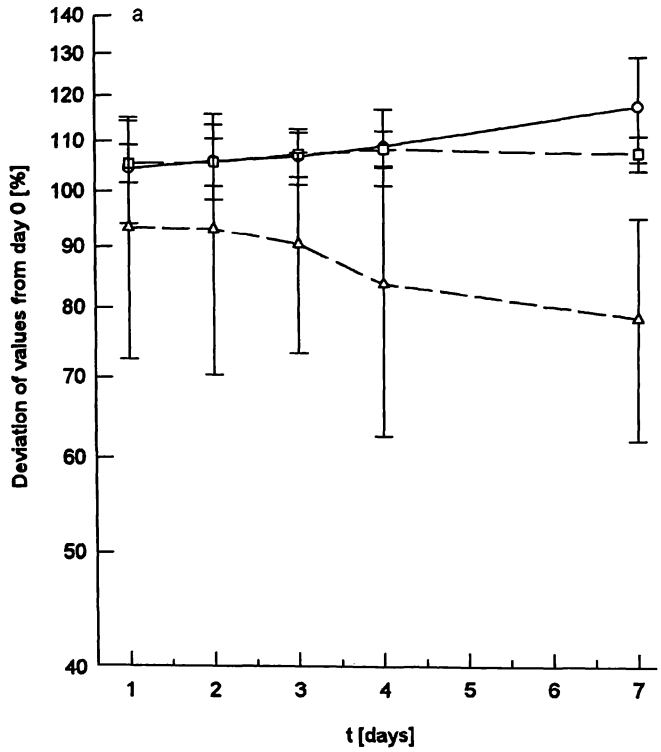


Fig. 2 Storage of centrifuged vacutainer® tubes at 9 °C. Deviation of the measurable quantities as mean and standard deviation in percentage of the initial value. From the top to the bottom: inorganic phosphate (circle) and lactate dehydrogenase (square).

lead to an increase of analyte concentrations in the serum. Haemolysis causes the release of erythrocytic constituents, which can result in increased values or in dilution leading to decreased values. Haemoglobin may also interfere in the measurement, e. g., in the photometric quantification of constituents. This influence also de-



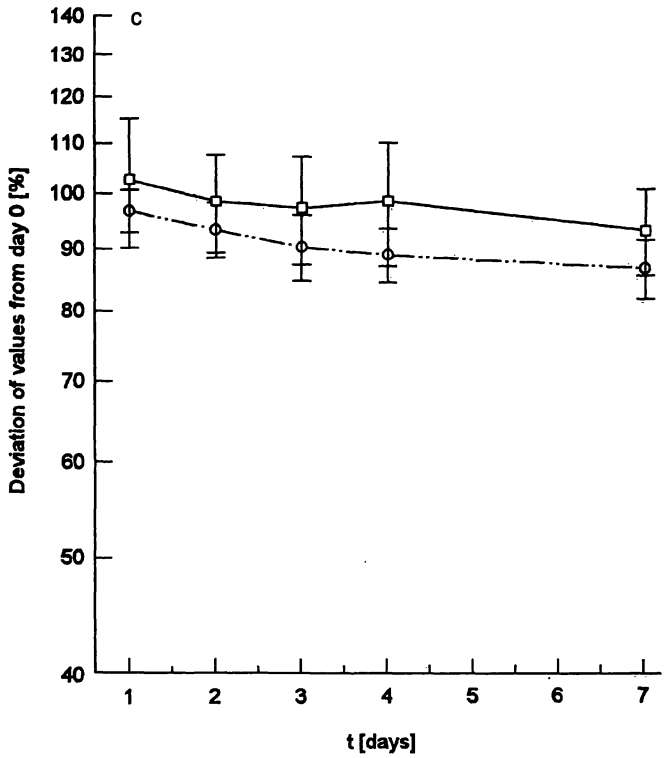


Fig. 3 Storage of centrifuged vacutainer® tubes at room temperature. Deviation of the measurable quantities as mean and standard deviation in percentage of the initial value.
a) Electrolytes, trace elements and metabolites from top to bottom: inorganic phosphate (circle), uric acid (square) and bilirubin (triangle).
b) Lipids and lipoproteins from top to bottom: triacylglycerols (hexagon), HDL-cholesterol (wedge) and LDL-cholesterol (rhomb).
c) Enzymes from top to bottom: aspartate aminotransferase (square) and creatine kinase (circle).

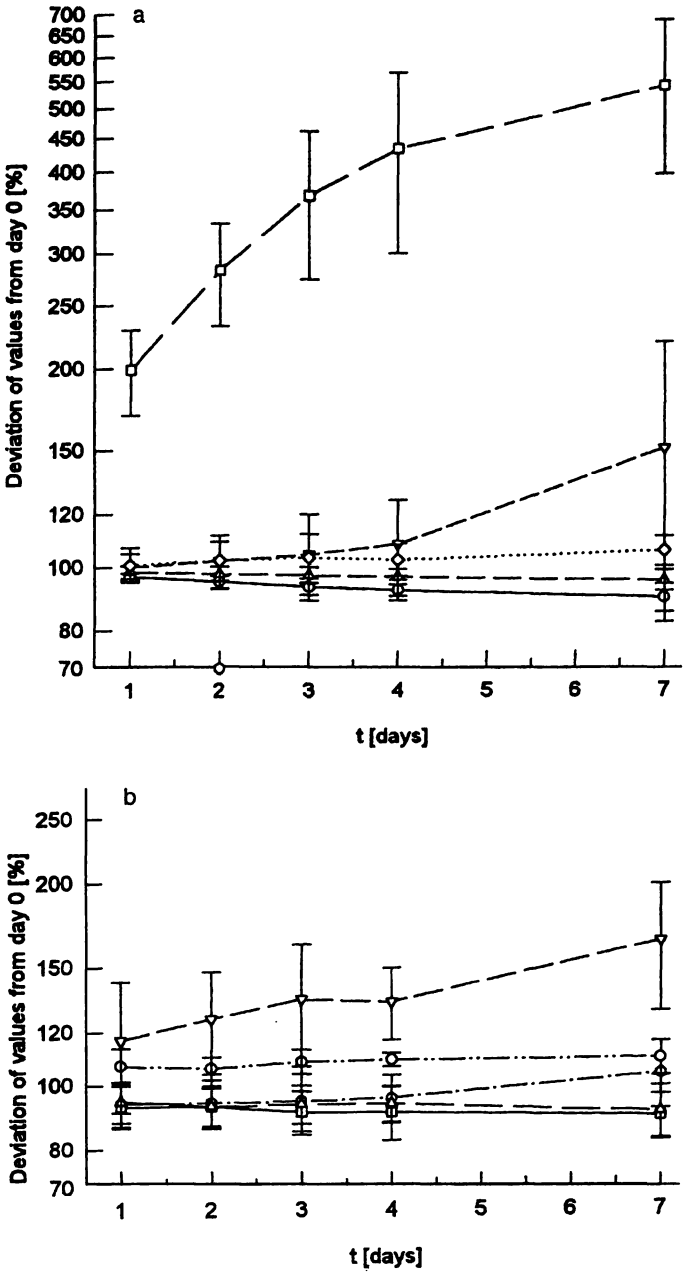


Fig. 4 Storage of not centrifuged vacutainer® tubes at 9 °C. Deviation of the measurable quantities as mean and standard deviation in percentage of the initial value.
a) Electrolytes and trace elements from the top to the bottom: potassium (square), inorganic phosphate (wedge), magnesium (rhomb), chloride (triangle) and sodium (circle).
b) Metabolites and enzymes from the top to the bottom: lactate dehydrogenase (wedge), uric acid (circle), creatinine (hexagon), α-amylase (triangle) and alkaline phosphatase (square).

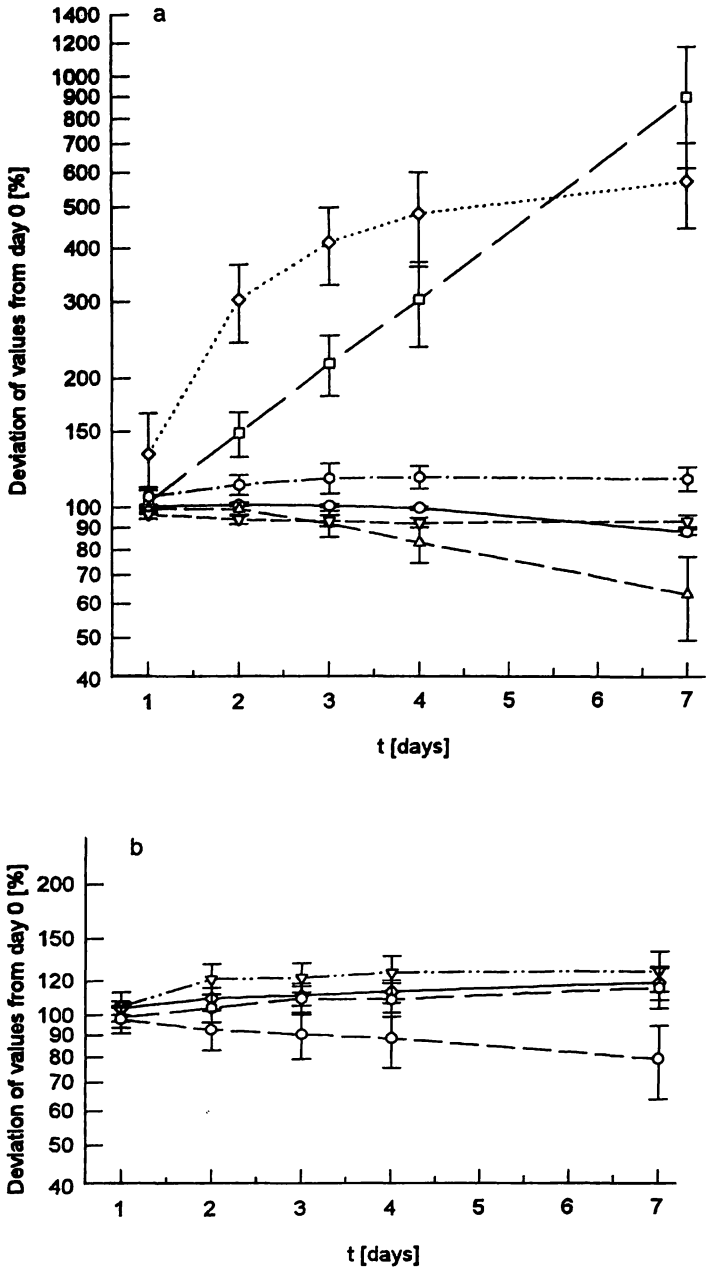


Fig. 5 Storage of not centrifuged vacutainer® tubes at room temperature. Deviation of the measurable quantities as mean and standard deviation in percentage of the initial value.
a) Electrolytes and trace elements from the top to the bottom: potassium (square), inorganic phosphate (rhomb), magnesium (hexagon), chloride (wedge), sodium (circle) and calcium (triangle).
b) Metabolites and lipoproteins from the top to the bottom: creatinine (wedge), urea (rhomb), HDL-cholesterol (hexagon) and LDL-cholesterol (circle).

pends on the main and the sub-wavelengths at which the measurement is performed. The above-mentioned interactions in the preanalytical phase are difficult to describe, because not all influences can be simulated in model experiments (2).

Electrolytes

Sodium, calcium and chloride concentrations decrease in whole blood because these low molecular mass substances enter the erythrocytes under the influence of their concentration gradients, namely 19 : 1 for sodium, 128 : 1 for calcium and 1.5 : 1 for chloride (8). Additionally, potassium and magnesium ions pass through the erythrocyte membrane due to the serum/erythrocyte ratio of 1 : 20 or 1 : 2.5. The slight increase in potassium after storage of centrifuged tubes is caused by the small number of erythrocytes on the surface of the polyester separation gel. Due to the temperature-dependent activity of the Na^+-K^+-ATPase (9), there are fewer shifts of sodium and potassium within the first 4 days, when whole blood is stored at room temperature than when it

is stored at 9 °C. The phosphatases in serum and whole blood cause a hydrolysis of phosphate esters, leading to an increase in the inorganic phosphate concentration. Since erythrocytes have an essentially higher concentration of phosphate esters, there is a greater increase in whole blood than in serum (10, 11).

Metabolites

No clinically relevant instability was observed in creatinine in serum over a period of one week. When whole blood was stored at room temperature, however, there was an increase in the measured concentrations of this analyte as a result of the non-specific formation of reactants, the so-called pseudocreatinines (12), for example, pyruvate from erythrocyte metabolism (13). Storage of whole blood at 9 °C had a slight adverse effect on the creatinine concentration indicating that this non-specific interference is influenced by temperature.

Urea in serum was found to be stable for one week. In the uncentrifuged tubes, cleavage of ammonia from basic amino acids may cause a slight interference with respect to the urease/glutamate dehydrogenase reaction. As a result, an apparent continuous increase in urea concentration in whole blood was observed at room temperature.

A small continuous increase in uric acid was observed in serum. In whole blood, haemoglobin may interfere in the measurement of this analyte, since haemoglobin interferes directly with the peridochrom method by spectral interference at 570 nm. In contrast, *Keller* (3) found a decrease in uric acid in plasma and whole blood, and *Sonntag* (2) reported a negative interference by haemolysis.

The instability of bilirubin in serum is caused by photooxidation. Additionally, in whole blood, the dichlorophenyl diazonium method (15) (a modified *Evelyn-Malloy* method) showed a positive interference by haemoglobin, in contrast to the *Jendrassik Gróf* method as reported by other authors (1–3, 16). Thus the decrease was compensated by the release of haemoglobin and there was no significant increase in the values.

Lipids

In serum, cholesterol, as well as HDL- and LDL-cholesterol concentrations, showed no significant shift during storage in the refrigerator over a period of 7 days. Other authors obtained the same results using polyethylene glycol 6000 as precipitating agent (17). In contrast, *Khan & Elkeles* (18) observed a decrease in the HDL-cholesterol concentration, using heparin manganese/dextran sulphate for precipitation. Storage of serum at room temperature produced adverse changes, i.e. an increase in the HDL- and a decrease in the LDL-cholesterol concentration, because of incomplete precipitation of LDL- and VLDL-lipoproteins (19, 20). The significant rise in cholesterol and HDL-cholesterol (and the decrease in LDL-cholesterol, which is partially compensated in whole blood but not in serum) in whole blood at room temperature resulted from an increase in cholesteryl esters accompanied by a smaller decrease in free cholesterol. This change seems to be a lecithin-cholesterol acyltransferase-dependent (EC 2.3.1.43) cholesterol transport out of blood cells (21). A significant drift in the triacylglycerol concentration was observed at room temperature. This apparent increase could be caused by cleavage of glycerol from phospholipids.

Enzymes

Inactivation of creatine kinase in serum increases with temperature and bright sunlight. This effect is not reversed by addition of sulphydryl (thiol) compounds. Oxidation of the sulphydryl groups in the active centre, which also causes an inactivation of the enzyme, is reversible. In an optimised assay, containing EDTA to reverse creatine kinase inhibition by calcium ions, and N-acetylcysteine to reactivate the sulphydryl groups, creatine kinase is reactivated by 97% (22) or to 99% (23). Thus, in contrast to earlier studies, reactivation of the creatine kinase activity by prior addition of thiol reagents to the sample is not necessary, but in the absence of such an addition we observed a greater decrease in enzyme activity with storage (24).

In whole blood, interference of adenylate kinase released by haemolysis of erythrocytes is inhibited by including diadenosine pentaphosphate and adenosine monophosphate in the reagent. We determined only a moderate temperature-dependent compensation of creatine kinase inactivation caused by other intermediates and enzymes from the erythrocytes, compared with methods using no inhibitor (25). In spite of the inhibitors added in the optimised assay, a high amount of adenylate kinase can also cause an increase in the catalytic concentration of creatine kinase (26).

In serum, a temperature-dependent decrease of aspartate aminotransferase was observed. At 9 °C, the enzyme was stable over 7 days. At room temperature, a slight continuous loss of activity occurred. Similar results have been described by *Cuccherini* (27) with a smaller decline in activity at lower temperatures. Samples with abnormal values showed a greater decrease. *Schmidt* (24) has also described a higher temperature-dependent decrease during storage. In whole blood, the measured aspartate aminotransferase activities did not increase

significantly over a period of 7 days. The increase was higher at 9 °C than at room temperature. This was obviously due to the greater stability of the enzyme. A non-significant increase due to haemolysis has been described by *Yücel* et al. (28). In model experiments other authors have observed a greater increase, due to haemolysis and 40-fold higher activity of aspartate aminotransferase in erythrocytes compared with serum (2, 25).

The alanine aminotransferase stability observed in serum corresponds to the data provided by *Williams* et al. (29), but the decrease at 9 °C and at room temperature was not significant over a period of 7 days. As far as whole blood is concerned, our results agree with the data confirmed by *Ruby* et al. (30), who found a greater loss of activity at room temperature than at 4 °C, but in our study the latter had no statistical significance. *Cuccherini* (27) has shown that the loss of activity in pathological samples is higher than in samples with a normal alanine aminotransferase activity.

Only the measurements of γ-glutamyltransferase after storage of whole blood at room temperature showed any significant alteration in enzyme activity. The distinct rise in haemolysis causes the release of glutathione, which inhibits enzyme activity at day 7 (31). *Persjin & van der Slik* (32) found interference by relatively high haemoglobin concentrations in this method.

Alkaline phosphatase showed a significant decrease after storage in whole blood. α-Amylase is stable in serum for one week, while in whole blood, the activity strongly decreased after 7 days at room temperature. The binding of *p*-nitrophenol to haemoglobin is highly dependent on the pH value of the test system. Therefore, the determination of enzyme activity based on the release of this substance is especially prone to interference at high pH values. This may explain a more marked decrease in the measured catalytic concentrations of alkaline phosphatase compared with that of α-amylase (2).

The slight but significant decrease in lactate dehydrogenase activity in serum at 9 °C is caused by an instability of the isoforms lactate dehydrogenase 3, lactate dehydrogenase 4 and lactate dehydrogenase 5 in the cold state (33). At room temperature, no significant change could be detected. In whole blood, a rapid increase in activity was observed because lactate dehydrogenase activity is 260 times higher in erythrocytes than in serum (34).

There were no clinically relevant differences in cholinesterase activity, either in serum over a period of 7 days or in whole blood over 4 days. Similar results were obtained by *Balland* et al. (35).

Conclusions

Our study confirmed that blood samples should generally be centrifuged as soon as possible. All measurable quantities examined were stable for four days in separated serum at 9 °C; however, values of inorganic phosphate, uric acid, bilirubin, triacylglycerols, HDL- and LDL-cholesterol and creatine kinase were not sufficiently stable when serum was stored at room temperature. If centrifugation is not possible within 24 h, chloride, inorganic phosphate, magnesium and γ-glutamyltransferase exceed the limits of stability when the samples are stored at room temperature. At 9 °C the latter are stable, but sodium, creatinine and alkaline phosphatase exceed the limits.

For the determination of potassium or lactate dehydrogenase in serum the storage of whole blood samples seems not to be acceptable.

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Dr. med. Michael Heins
Institut für Klinische Chemie und
Laboratoriumsdiagnostik
Heinrich-Heine-Universität Düsseldorf
Moorenstraße 5
D-40001 Düsseldorf
Germany